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NUTRITION REVIEWS

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NUTRITION REVIEWS

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EARLY EXPERIENCES WITH CHOLINE—A RETROSPECT

The recognition of choline as a dietary factor which prevents pathologic fatty changes in the liver was a direct outcome of the discovery of insulin. My interest in liver fat was first stimulated in the spring of 1921 when Banting and I noted the large yellow livers of our untreated, completely depancreatized dogs. This finding had been originally described by J. von Mering and O. Minkowski (*Arch. f. exp. Pathol. u. Pharmakol.* **26,** *371 (1889)*). The restoration of this diabetic fatty liver to a normal condition by administration of insulin was first reported by the Toronto group in 1922 (F. G. Banting *et al., Tr. Roy. Soc. Canada* **16,** *Sect. V, 39 (1922)*). Banting and I fully appreciated, as our notes made at the time reveal, that prolonged observations on completely depancreatized dogs treated with insulin might provide fascinating new facts, but the development of a large fatty liver, which was *independent* of the supply of insulin, was quite unexpected. This was noted independently and at about the same time by F. N. Allan, D. J. Bowie, J. J. R. Macleod, and W. L. Robinson of Toronto (*Brit. J. Exp. Path.* **5,** *75 (1924)*), and by N. F. Fisher of Chicago (*Am. J. Physiol.* **67,** *634 (1924)*).

The most obvious abnormality in these animals was the lack of the pancreatic enzymes and support of this as the causative mechanism was provided by the finding that when beef pancreas was included in the diet, which consisted of lean meat and cane sugar, the fatty livers did not develop. The next step seemed obvious, *i.e.*, to identify the factors in the pancreas which were responsible for the protection of the liver. According to Dr. Jacob Markowitz, who was working in the laboratory at that time, crude preparations of pancreatic lipase and of trypsin were found to be beneficial. But this finding was not published.

Three or four years later attention was directed toward an entirely different component of the pancreas as a result of the work of two graduate students, Hershey and Soskin. James M. Hershey, the Chief Chemist of the Insulin Committee in Toronto, was working toward an M.A. degree in the Department of Physiology. Impressed by evidence which at that time seemed to indicate that phospholipids were concerned with the transport of fat, he formulated a hypothesis that a failure in fat metabolism in the depancreatized dogs, rather than a defect in digestion, might be responsible for the large fatty livers of these insulin-treated animals. He therefore added crude egg-yolk "lecithin" to the daily ration of a number of insulin-treated depancreatized dogs, and found that apparently healthy conditions could be maintained for a long period of time. Dr. Samuel Soskin, who was then a recent medical graduate of Toronto and working for his PH.D. in Physiology, joined Hershey in this problem and his knowledge and experimental skill added a great deal to this partnership. It was at this stage of the study, 1929, that Dr. J. J. R. Macleod left Toronto and returned to his alma mater, the University of Aberdeen, to occupy the Chair of Physiology. When I assumed direction of the Department of Physiology in Toronto in 1929, Professor Macleod was at great pains to warn me that he was not convinced that Hershey's and Soskin's findings were new and he strongly advised me to watch the experiments and to assure myself by personal observation that the liver damage was not due to lack of some well-recognized dietary constituent which might be contained in the crude "lecithin."

The observations were therefore extended for more than a year before the first report of this work was made by Hershey from my department (*Am. J. Physiol.* **93,** *Proceedings, 657 (1930)*).

Experiments on depancreatized dogs were not only time-consuming, but the variability of the results made the use of large numbers of animals imperative. The limitations of space, and the cost of rations and salaries for attendants, made it difficult to maintain the experiment with dogs at that time. With Miss Mary Elinor Huntsman, now Mrs. Colin Mawson, we placed rats on a high-fat (incidentally a low-protein) diet and found that in the relatively short period of three weeks, fatty livers developed. With these new test animals it was possible to show that lecithin, purified by the cadmium chloride method of P. A. Levene and I. P. Rolf (*J. Biol. Chem.* **46,** *193 (1921)*), exerted a very definite effect on the deposition of liver fat in the rat (C. H. Best, J. M. Hershey, and M. E. Huntsman, *J. Physiol.* **75,** *56 (1932)*). These were very exciting days in the laboratory and within a month we were able to publish a note identifying choline as the active constituent of the lecithin molecule. It was shown that sodium beta-glycerophosphate, calcium alpha-glycerophosphate, sodium oleate and ethanolamine were inactive. It was noted in the same report that betaine has an effect on liver fat similar to that of choline (Best, Hershey, and Huntsman, *Am. J. Physiol.* **101,** *7 (1932)*; Best and Huntsman, *J. Physiol.* **75,** *405 (1932)*). In 1933 we reported that choline was effective in preventing the development of the fatty livers in the insulin-treated depancreatized dogs (Best, G. C. Ferguson, and Hershey, *Ibid.* **79,** *94 (1933)*).

Unexpected results in one of the experiments with rats indicated that protein is an important factor in these lipotropic studies (C. H. Best and M. E. Huntsman, *J. Physiol.* **83,** *255 (1935)*). This observation, which was communicated to Professor H. J. Channon of Liverpool, who had spent a few months working with us, led to a detailed examination of the lipotropic effect of various proteins and amino acids. During this search, which was particularly active in Professor Channon's laboratory (Channon and H. Wilkinson, *Biochem. J.* **29,** *350 (1935)*; A. W. Beeston and Channon, *Ibid.* **30,** *280 (1936)*), the report of H. F. Tucker and H. C. Eckstein (*J. Biol. Chem.* **121,** *479 (1937)*) from Ann Arbor was published and solved in very large part the problem of the lipotropic constituent of protein by showing that methionine exerts a strong action.

Dr. Earle Willard McHenry, who had worked with me for several years on the study of histamine and histaminase, became interested in the cause and cure of fatty livers. In 1941, working with the late Dr. Gertrude Gavin, he discovered the lipotropic activity of inositol (*J. Biol. Chem.* **139,** *485 (1941)*). Later it was shown by James M. R. Beveridge (*Science* **99,** *539 (1944)*), now Professor of Biochemistry at Queen's University, Kingston, Canada, that the lipotropic effect of inositol is negligible when diets containing fat are used.

It is very interesting that a number of people who have been associated, more or less closely, with the Toronto laboratories, have continued their interest in lipotropic phenomena. To mention a few of them: Professor Arnold De Merritt Welch who, in 1934, was working toward a postgraduate degree in the Department of Pharmacology, University of Toronto, and his wife, Mary Scott Welch, who was registered for an M.A. in the Department of Physiology, became curious about certain aspects of the choline problem. Their subsequent studies of lipotropic phenomena, although done quite independently of our group, doubtless had some part of their origins in these earlier contacts. Another name prominent among those active in this branch of nutrition is that of Thomas Hughes Jukes, who was registered in the

School of Graduate Studies in the University of Toronto, and was taking our course in Advanced Physiology at a time when the choline field was developing rapidly. Professor I. L. Chaikoff was a pupil of Professor J. J. R. Macleod and his interest in diabetes and fat metabolism dates from the time when he took his PH.D. in Toronto in 1924. Dr. Henry Borsook, whose studies in this field have been less directly related to the lipotropic aspects of choline than to its function as a methyl donor, was a classmate of mine for four years, but that was before the "lipotropic" era.

I have been asked to limit this short article to the early stages of the choline story, so I can only mention very briefly the brilliant light thrown on this field by du Vigneaud, Stetten, Griffith, György, Handler, Salmon, Engel, Copeland, Artom, Daft and Sebrell, Himsworth, and many others including my colleagues C. C. Lucas, W. S. Hartroft, Jessie H. Ridout and E. A. Sellers. A great many of the interesting features of this subject have frequently been vigorously debated in friendly fashion at the Josiah Macy Jr. Conferences on Liver Injury. I have found that many of the substances in which I have been most interested—insulin, choline, heparin and the fat-mobilizing effect of anterior pituitary extracts which Dr. James Campbell and I were perhaps the first to demonstrate clearly—all have their significant effects on fat storage or transport.

Looking back from the vantage point which we have now gained it is apparent that the nature of the substances responsible for the lipotropic activity in minced-beef pancreas might have been effectively attacked in any one of several ways. If the protein fraction of the pancreas had been fed to rats on diets rich in fat, the lipotropic activity of methionine-containing protein might have been found earlier. If a more thorough study of the administration of enzymes obtained from the pancreas had been carried out in the insulin-treated depancreatized dogs, the indirect lipotropic action of these substances, later so effectively elucidated by Chaikoff and his colleagues, would presumably have been recognized. I have described very briefly the route which was actually followed in the recognition of the lipotropic activity of choline, betaine, methionine and inositol. It has become more and more apparent that choline provides an essential part of the pathway which methionine and betaine must use in the production of their lipotropic effects, but a discussion of these and the many other fascinating interrelationships involving the sources of the methyl group, vitamin B_{12}, etc., would take us too far afield. It is a great satisfaction that this aspect of fat metabolism developed by many laboratories over the past twenty-one years has been such a useful tool with which to reveal new vistas in physiology, biochemistry and pathology.

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IRON DEFICIENCY IN CONGENITAL HEART DISEASE

The hypochromic, microcytic anemia characteristic of iron deficiency in infants and young children is ordinarily accompanied by a moderate or severe decrease in the hemoglobin concentration below levels considered normal for the age of the patient. That iron deficiency does occur in infants with cyanotic congenital heart disease without such decreases has been pointed out by A. M. Rudolph, A. S. Nadas, and W. H. Borges (*Pediatrics* **11,** *454 (1953)*).

Seven cyanotic children aged 5 months

to 4 years had hemoglobin concentrations of more than 10 g. per cent and erythrocyte counts ranging between 5.2 and 9.3 millions. Their red cells were both microcytic and hypochromic. They all had symptoms commonly associated with anemia such as irritability, anorexia, poor weight gains, or attacks of dyspnea. Treatment with iron as ferrous sulfate supplying 30 mg. of iron daily resulted in a mild reticulocytosis in ten to fourteen days, subsequent clinical improvement, and an increase in hemoglobin levels with disappearance of the microcytosis and hypochromia.

This condition has been termed "relative anemia" by these authors. However, in view of the low oxygen saturation present in the blood of these patients and the necessity of their maintaining hemoglobin concentrations at abnormally high levels to prevent tissue anoxia, it differs in no significant way from the anemia of iron deficiency in normal children.

The return of symptoms similar to those noted before the onset of therapy often ensued after treatment was accomplished. These symptoms were associated with increases in the hematocrit to values above 70 per cent, at which level great increases in the blood viscosity occur, resulting in poor tissue oxygenation. Consequently the administration of unlimited amounts of iron to infants having cyanotic congenital heart disease is not without potential dangers.

The development of iron deficiency anemia in congenital heart disease was observed in a group of infants followed from birth. Three noncyanotic infants and 2 of 3 mildly cyanotic infants had hemograms demonstrating the normal decrease in hemoglobin concentration and erythrocyte count during the first few months of life. The 2 severely cyanotic infants studied failed to show this pattern and their erythrocyte counts gradually increased while their hemoglobin concentrations were maintained at or near the levels present at birth and then fell to "normal" concentrations during the second half-year, giving rise to both microcytosis and hypochromia. The need for an increased circulating hemoglobin mass in these cyanotic infants puts a severe stress on their endogenous and dietary iron supplies. As has been recently pointed out (*Nutrition Reviews* **11,** *163* (*1953*)), stabilization of the hemoglobin concentration in the first few months of extrauterine existence appears to depend upon the oxygen saturation of the blood. When this is abnormally decreased, as in cyanotic congenital heart disease, the requirements for circulating hemoglobin exceed the available supplies of iron much more rapidly than in the normal infant.

This syndrome of "relative anemia" in infants with cyanotic congenital heart disease furnishes another example of the specificity of microcytosis and hypochromia in the diagnosis of iron deficiency in human subjects.

WATER FLUORIDATION AS A PUBLIC HEALTH MEASURE

In a recent symposium, F. S. McKay (*Am. J. Pub. Health* **43,** *700* (*1953*)), who was one of the earliest investigators to describe "mottled enamel" and note that it was caused by a water-borne agent, has reviewed the early history of the relation of fluorides to dental health. In 1916, McKay, with G. V. Black, presented a detailed description of the disfiguring condition of "mottled enamel" as seen in 6873 individuals from 26 communities (*Dental Cosmos* **58,** *477*, *627*, *781*, *894* (*1916*)). In 1931 the etiologic agent was found to be excessive amounts of fluorides in the communal drinking water. Unfortunately, its relation to reduced caries incidence was not recognized until

the United States Public Health Service took up a detailed survey of fluorides in water supplies, the relation to caries incidence, and the optimal fluoride levels for caries protection without disfiguring fluorosis.

In the present review, McKay confidently states, "No problem in epidemiology has ever had so much supporting evidence as has the action of fluoride in the prevention of dental caries." In a study in one community which had used naturally fluoridated water for seventy years, he reported that in 511 persons, with an average age of 25 years, 36 per cent of the subjects were totally free of caries. The average number of filled and carious teeth was 2.6, and the average number of teeth lost was less than 0.02 per person. Carious lesions were particularly infrequent in anterior teeth and were characteristically limited to the pits and fissures of the posterior teeth. He described another survey made in 1947 of the Salida, Colorado, area where the water contained 1.3 parts per million (p.p.m.) of fluorides. Forty-one per cent of the 741 school children studied were entirely free of caries, and the caries incidence rate was less than one-fifth of a corresponding study group in Madison, Wisconsin, where the water is fluoride-free. The investigators in this survey were quoted as observing "that mildly fluorosed teeth had a lustre, sheen, and beauty peculiar to themselves."

McKay quotes from recent studies which show that there are still numerous communal water supplies in the United States which contain in excess of 3.0 p.p.m. of fluorides (*J. Am. Water Works Assn.* **44,** *553* (*1952*)). Some of these high levels have existed for seventy years or more, so that the lifelong inhabitants of the areas concerned have been exposed to these levels throughout each decade of their life. He challenges the opponents of fluoridation to make a scientific study of the inhabitants of one of these high fluoride areas. In this way, final evidence could be obtained as to whether such high levels of fluoride intake throughout the lifetime of an individual caused or increased the susceptibility to any other pathoses than mottled enamel. McKay strongly believes that except for mottled enamel even levels far beyond the recommended level of 1 p.p.m. in the drinking water are entirely innocuous when consumed throughout the entire life cycle.

In the same symposium, H. T. Dean (*Am. J. Pub. Health* **43,** *704* (*1953*)) points out that fluoridation of water is an accepted public health procedure and that fluoridation is passing from the basic research stage to widespread public application. In a summary of the latter point, Dean points out, "as of May 1st, 1953, there were 14,300,000 residents of 769 communities served by 407 separate water supplies using domestic water supplies which are fluoridated. In addition, there are 15,800,000 people served by 353 water supplies where the procedure has been approved but actual fluoridation has not as yet begun." He bases his statement that water fluoridation is an acceptable health procedure on the evidence from (1) surveys of caries incidence in communities throughout the world where there are naturally occurring water-borne fluorides, (2) independent field reviews of dental caries experience in Grand Rapids, Michigan, Newburgh, New York, and Brantford, Ontario, where the water supplies have been artificially fluoridated at levels of around 1 p.p.m. since approximately 1945, and (3) lack of evidence that fluoride ingestion at the 1 p.p.m. level recommended for public health application has any deleterious influence.

In another of this series of presentations, E. A. Schisa (*Am. J. Pub. Health* **43,** *710* (*1953*)) discusses the role of the public health nurse in the community water fluoridation program. She stresses the need for the public health nurse, "the spearhead of the public health program," to be well-informed on the experimental and epi-

demiologic data, and the need for the public health nurse to interpret carefully to inquiring individuals the possibilities and limitations of fluoridation in the partial control of tooth decay. Although this was not mentioned, it should also be pointed out that there is a real need for physicians and dentists to be well-informed and capable of presenting the facts on fluoridation without bias.

F. J. McClure (*J. Am. Dietet. Assn.* **29,** *560 (1953)*) has reviewed the fluoride content of foods and drinking water and has made some interesting calculations of the probable range of total daily fluoride consumption by children of different ages. Values are presented for fluoride content of a wide range of foodstuffs from fruits, vegetables and tubers, to cereals, meat, fish, eggs and milk. Particularly notable for their high fluoride content are tea and seafoods. Tea leaves were reported to contain 75 to 100 p.p.m. of fluoride, with a cup of tea containing approximately 0.12 mg. of fluoride. Seafoods, as eaten, contained from 4.2 p.p.m. for canned salmon to 27 p.p.m. for fresh mackerel. Fruits, on a fresh weight basis, were on the average lower than other food categories, with levels as low as 0.02 p.p.m. for some apricot samples to 0.70 p.p.m. for some apple samples. Cereals were on the whole fairly low. Milk was also found to be low, with values reported from 0.07 to 0.30 p.p.m. It is noteworthy that there appeared to be no appreciable increase in the fluoride content of milk when fluorides were added to the cow's ration or drinking water. Vegetables and tubers, on a dry weight basis, varied widely within the group and within individual foods. For example, the content of peas was listed as varying from 0.60 to 6.69 p.p.m., a range that is compatible for the entire vegetable group. The comment was made that the fluorides naturally present in the soil or as added by chemical fertilizer had little or no influence on the fluoride content of edible plant produce.

In McClure's summary of the estimated daily intake of fluorides from food and from drinking water, the assumptions were made that the drinking water contained 1 p.p.m. of fluorides and that the dry substance of food contained 0.1 to 1 p.p.m. of fluorides. The estimated fluoride intake for the 1 to 3 year old children ranged from 0.417 to 0.825 mg. per day and increased progressively until values for 10 to 12 year old children were estimated to be 0.866 to 1.725 mg. per day. These values were uniformly under 0.1 mg. per kilogram of body weight.

McClure summarized some of the pertinent data on the effect of fluorides on health status. At levels of more than 1.5 p.p.m. of fluoride in the drinking water during periods of tooth development, mottled enamel was observed as an effect of the ingestion of these amounts of fluorides. However, in areas where mottled enamel was prevalent, height and weight data, as well as bone fracture experience were comparable to nonfluoride areas. Calcification of the carpal bones of children, 6 to 13 years of age, was normal in areas where the drinking water contained 4.0 to 4.5 p.p.m. of fluorides. No evidence of kidney damage has been observed in high fluoride areas. In support of the latter opinion, he cites comparable mortality rates due to nephritis for the period from 1945 to 1949 in Stevens Point, Wisconsin, where the water contained 0.2 p.p.m. of fluorides, and Green Bay, Wisconsin, where a level of 2.5 p.p.m. of fluorides is naturally provided. Likewise, there was a decrease in mortality due to nephritis in Sheboygan, Wisconsin, in the five years after fluoridation was begun as compared to the previous five years.

McClure reviews an unpublished survey made by a group of the United States Public Health Service investigators as follows: "Among a group of 114 adults whose age averaged forty-three years for men and forty-six for women and who had been exposed to as much as 8.0 p.p.m.

fluorine in their drinking water for an average of twenty years, there were no systemic complaints which could be related to the fluoride water, and in only seven individuals were there signs of an incipient skeletal change apparent by X-ray examination." This survey obviously is one of the type and with the purpose proposed by McKay above. It is noteworthy that no systemic complaints were noted in these individuals even though they had consumed water bearing as much as eight times the optimal level continuously for an average of twenty years. The significance of the incipient skeletal changes in 7 individuals cannot be determined on the basis of the above statement. Obviously it was insufficient to have caused any abnormality that would be noticed subjectively or detected upon physical examination.

McClure drew attention to one fact which merits thorough study. Upwards of 35 per cent of the United States population does not use a commercial water supply. The problem of an adequate fluoride intake for these individuals has not been solved. It is a more difficult problem because there is presently no suggestion as to how a carefully controlled, safe source of fluoride can be introduced into individual homes.

The progress in the fluoride problem during the past thirty-five years, beginning with an epidemiologic survey of an obnoxious public health problem, to epidemiologic surveys of a beneficial phase of the subject, to field testing of the agent and finally widespread adoption of water fluoridation as a public health measure is dramatically brought out in the above papers. As McKay, Dean and McClure sagaciously point out, additional studies should be conducted with enthusiasm. It is their expectation that those studies would be likely to indicate ways and means to improve the effectiveness of fluoride ingestion and to increase the availability of fluorides to larger percentages of the population and are highly unlikely to reveal facts which would raise a question as to the advisability of controlled water fluoridation.

SURVIVAL ON RATIONS OF LOW CALORIC CONTENT

The experiments carried out at the Quartermaster Climatic Research Laboratory, Lawrence, Massachusetts, and reported by M. Quinn, C. R. Kleeman, D. E. Bass, and A. Henschel (*Office Quartermaster General, Environmental Protection Branch, Reports Nos. 201 and 205 (1953)*) arose out of the very practical necessity to supply information needed in approaching more closely an "ideal" survival ration. Obviously, this will always be a compromise among the criteria offered by logistics, food technology, and the science of human nutrition. However, the machinery of the body and its alterations in a variety of survival conditions on a given dietary regimen must receive priority considerations, serving as the guide to manufacture of the ration and to the efforts to supply it to men in the field.

While the problem of survival rations has confronted the Quartermaster Corps for many years, there still remain important nutritional aspects to be clarified. The studies by Quinn *et al.* (*loc. cit.*) were designed to do just that and they have succeeded in reducing the area of uncertainty. Their experimental situation has parallels in nature. At the same time it may be well to point out that the findings have relevance not only for military and disaster situations involving several days of subsistence on a drastically lowered caloric intake (and on limited water), but also increase the general fund of scientific knowledge concerning energy, protein and water metabolism and body composition.

It is inherent in the nature of situations in which survival itself becomes the goal that only minimal amounts of food and, frequently, of water, can be made available. Consequently, caloric balance cannot be maintained and the possibility of maintaining water balance is questionable. The principal problem in the design of a survival ration is thus reduced to furnishing nutrients which will minimize, if possible, the breakdown of body protein without increasing the obligatory urine volume.

The specific hypothesis, suggested by results of experiments on both animals and man (carried out elsewhere) and put to test in these studies, was that under conditions of semistarvation survival the addition of protein to a protein-free ration would spare the breakdown of body protein and would not increase the minimal water requirements. Ten young volunteer enlisted men served as test subjects. They lived in a constant-temperature chamber for twenty-two days. During the control phase (thirteen days) a nutritionally adequate diet was consumed, ranging in energy value from 2800 to 3300 calories. During the nine-day experimental period the subjects were divided into two groups of 5 men each on the basis of body weight and fatness. The nonprotein diet provided 950 calories, with only a trace of protein, 52 g. of fat and 116 g. of carbohydrate. In the protein diet, similar in caloric value and fat content, there were 70 g. of carbohydrate and 43 g. of protein (egg albumen). Drinking water was restricted to 800 ml. daily. The diet was synthetic, and the food and water allotments were consumed in four feedings. The subjects were semiambulatory throughout the experimental period.

The working hypothesis had to be rejected. The added protein did not improve nitrogen balance and served, under the given conditions, only as a source of energy. It was the protein-fed group which manifested a greater loss of water, lost largely at the expense of the extracellular water. Negative water balances were present in both groups. In the protein-fed group the larger water loss was due to the increased excretion of protein metabolites. Also, ketonuria was confined almost exclusively to the protein-fed group. There were no appreciable differences in electrolyte balances between the two groups.

In the second experiment, carried out under identical conditions except for an increase of water intake (mean of 1750 cc. daily), the over-all picture remained about the same. Even with the increased water intake, the added dietary protein did not spare the breakdown of body protein and there was no significant difference in nitrogen balance. The losses of weight, fat and water in the two groups were similar. It should be noted that there was still a negative water balance, even though dehydration was not a prominent feature of this type of semistarvation. Again, ketonuria was observed and was limited to the protein group. There was suggestive evidence that the protein group lost less potassium but more sodium and chloride. In practical terms, neither the replacement of some carbohydrate by protein in a low-calorie ration nor the addition of water resulted in an appreciable difference in the metabolic pattern, the ingested protein being utilized primarily as a source of fuel.

Having considered the differences between the two groups, we may turn our attention to the common effects of subsistence on drastically reduced caloric intake for a little over one week. To simplify matters, only the values for the first experiment will be considered here, with special emphasis on the composition of the body. While no fully adequate methods for the study of body composition *in vivo* are available, the experimental results indicate clearly that even moderate weight losses are anything but a simple matter. In these studies the average body weight loss was 4.8 kg. (7.3 per cent of the initial weight). This loss was partitioned into body fat,

estimated from skinfolds (J. Brozek and A. Keys, *Brit. J. Nutrition* **5,** *194* (*1951*)), and the remaining "lean body mass" (essentially muscle and viscera, bone mineral and water). While the absolute losses in the two compartments were identical (2.4 kg.), there was a ten-fold difference when the decrements were expressed as percentages of control value (40 per cent for fat, 4 per cent for "lean mass"). The cumulative negative nitrogen balance amounted to 58.2 g., equivalent to about 364 g. of protein (1.1 kg. of lean muscle). Estimations of extracellular water, measured as the "chloride space," indicated a loss of 1.4 liters. These calculations, made from the author's data, suggest that fat, protein and extracellular fluid would account for the observed weight loss.

Total body water was not determined directly, but was calculated as 72 per cent of the lean body mass during the control period. The *changes* in total body water were calculated as external water balance minus water of oxidation from body fat and body protein. The difference between the "total" and "extracellular" water yielded the estimate of "intracellular" water. The assumptions which have to be made in such calculations are rather overwhelming and the authors are aware of the potential criticism of employing the particular method for measuring the extracellular water. They emphasize that they were concerned with the changes in this body compartment, not with the definitions of its boundaries.

Of the negative "balance" of total body water, as defined above, 63 per cent is accounted for by the extracellular and 37 per cent by the intracellular water compartment. In view of the theoretic limitations of the methods applied, the accuracy of calculated weight loss (from fat, protein and total body water) as compared with the observed weight loss is remarkable. A similar experience was had in the second study, in which the water intake was substantially increased. Data are presented on urine, blood and plasma volume, losses of serum electrolytes, urinary excretion of ketones (in the protein group), creatinine excretion, hemoglobin, hematocrit, and plasma protein, plus determinations of urinary ammonia and titratable acid (in the second study).

In summary, the investigations reviewed above have not only answered the specific question of the use of protein in a survival ration supplying about 900 calories per day, but have resulted in the addition of fundamental data on human nutrition. It is hoped that the material will be published in the form of journal articles and thus become more generally accessible than are the unclassified reports of the Environmental Protection Branch of the Office of the Quartermaster General.

The studies have been concerned with the type of "static" survival, characterized by minimal physical activity and protection from exposure to the elements. Even under these conditions some deterioration of physiologic fitness and performance capacity will take place. It is these aspects which will assume central importance in case of "dynamic" survival, involving escape and evasion. This type of survival has not been adequately and systematically investigated in the past, although much attention has been devoted to acute starvation.

TAGGED ALBUMIN AND GLOBULIN METABOLISM IN CIRRHOSIS

The increasing availability of radioactive isotopes and greater knowledge of the technics of measuring isotopic materials have recently stimulated several investigations of protein metabolism. Using albumin and globulin tagged with radioactive iodine several workers have attacked the problems of protein synthesis and degradation, dis-

tribution of albumin in body fluids, and protein transfer across tissue membranes.

Early studies of the use of iodine-tagged albumin in human subjects were those of K. Sterling (*J. Clin. Invest.* **30,** *1228, 1238 (1951)*), who found that patients with cirrhosis exhibited a longer half-time of disappearance of the labeled albumin than did normal subjects. Mean half-time in 11 patients with cirrhosis with and without ascites was 12.9 days compared with 10.5 days in 21 normal control subjects. Turnover rate in grams per day was 11.9 in cirrhotic patients and 17.2 in normal subjects, or, expressed differently, 5.49 per cent of circulating albumin was metabolized daily in cirrhotic patients versus 6.7 per cent in normal subjects. Slower turnover rates appeared to correlate with low serum albumin concentrations, but did not necessarily reflect the presence or absence of ascites. By contrast, in 2 patients with hypoalbuminemia without liver disease, turnover rates were normal.

M. P. Tyor and D. Cayer (*South. Med. J.* **45,** *141 (1952)*; *Nutrition Reviews* **10,** *268 (1952)*) in similar studies found slower albumin disappearance rates only in patients with ascites. These results, however, were based on determinations of disappearance from the vascular compartment at forty-eight to seventy-two hours whereas Sterling's determinations were made over a fourteen-day period. This difference in the duration of study may be significant in view of the reports of other investigators (*see above*) in regard to the time required for equilibration of tagged albumin between plasma and ascitic fluid. In further studies Tyor, J. K. Aikawa, and Cayer (*Gastroenterology* **21,** *79 (1952)*) have been unable to find a difference in the rate of disappearance of tagged albumin from the plasma in the first day in cirrhotic subjects as compared to patients with other disorders. There was, however, a significantly lower urinary excretion of inorganic iodide in the patients with hepatic disease in the twenty-four hours following ingestion. Although the latter finding suggests decreased albumin catabolism, the brief period of study renders this work relatively inconclusive. In addition, although 7 of the 9 cirrhotic patients had ascites, no details of differences in urine iodide excretion in the presence or absence of ascites were reported.

F. K. Bauer, W. H. Blahd, and M. Fields (*J. Clin. Invest.* **32,** *553 (1953)*) studied 2 patients with cirrhosis and one with hemochromatosis, all with ascites, after tagged albumin was injected both intravenously and intraperitoneally. Following injection by either route a constant rate of exchange between the two compartments occurred at approximately the fifth day. Approximately the same time of establishment of equilibrium has been found by W. J. Eisenmenger and R. J. Slater (*Ibid.* **32,** *564 (1953)*), who observed equilibrium in two and one-half to six days in the majority of patients when either tagged albumin or globulin was used. Certain patients, however, required longer periods for equilibration when the protein was given intraperitoneally. In another study S. A. Berson, R. S. Yalow, S. S. Schreiber, and J. Post (*Ibid.* **32,** *554 (1953)*) found that distribution of tagged albumin in nonedematous subjects occurred in four to seven days whereas the presence of ascites usually lengthened the time necessary for distribution.

Appropriate studies have been performed to determine whether disappearance or distribution of the iodine label actually reflected the metabolism of the whole albumin molecule. Bauer and his colleagues (*loc. cit.*) carried out dialysis, trichloracetic acid precipitation and paper chromatography and found the iodine in the protein component of the serum. By the use of specific antisera and fractionation they further demonstrated the iodine to be contained in the albumin fraction. Berson and his coworkers (*loc. cit.*) and Eisenmenger and

Slater (*loc. cit.*) also demonstrated by the use of electrophoresis that the iodine remains in the albumin molecule.

The rate of catabolism or degradation of albumin has been determined in normal and cirrhotic subjects by several other groups with results somewhat at variance with those reviewed above. Berson and co-workers (*loc. cit.*) found degradation rates of 3.3 to 4.5 per cent per day in noncirrhotic subjects, but gave no figures for patients with liver disease. Eisenmenger and Slater (*loc. cit.*) reported the half-life of albumin to be nine and one-half to eleven and one-half days in normal and in cirrhotic subjects without ascites. Those with ascites were reported to have slower rates of degradation, although no figures were given. These workers suggested, however, that albumin in the peritoneal cavity is probably not subject to catabolism and state that correction for this fraction of the albumin results in normal half-life figures. The discrepancies between these results and those obtained by Sterling and by Tyor and Cayer (*see above*) may be explained at least in part by the finding of Berson and his associates that differences in degradation rate were obtained using albumin prepared in their own laboratory and albumin prepared commercially. The details of these studies were not given in this preliminary report and consequently the magnitude of this variation is not available.

The distribution and metabolism of radioiodine-tagged gamma globulin has also been studied by Eisenmenger and Slater (*loc. cit.*). Half-life of this protein in normal subjects was found to be eleven and one-half to nineteen days, but in cirrhotic patients both with and without ascites a half-life of six to thirteen days was observed. Similar results were obtained by W. P. Havens, J. Dickensheets, J. N. Bierly, and T. P. Eberhard (*J. Clin. Invest.* **32,** *573 (1953)*), who found the half-life of gamma globulin in normal subjects to be ten and six-tenths to sixteen and six-tenths days (average thirteen and one-half) and five and two-tenths to ten and eight-tenths days (average seven) in patients with liver disease. Distribution or rate of disappearance of radioactivity from the blood was approximately the same in both groups in the latter study, being 16 to 25 per cent for the first four hours and 25 to 50 per cent in twenty-four hours. Measurement of radioactive iodine appearing in the urine in the first four days after globulin injection was also carried out by Havens and his colleagues. In 2 control subjects and 1 patient with cirrhosis 30 to 60 per cent of the administered dose appeared in the urine in this period. Again, these preliminary reports do not yield details, but it would appear that gamma globulin breakdown is more rapid in cirrhotic than in normal subjects. This finding is of considerable interest in patients with liver disease, for, in view of the high incidence of hypergammaglobulinemia in patients with cirrhosis, increased metabolic decay as observed in these studies suggests a very high rate of gamma globulin production in order to maintain the high plasma levels observed.

The studies reviewed present considerable data on the important problem of protein metabolism in patients with liver disease. However, it cannot be concluded that albumin catabolism is decreased as a reflection of or compensation for reduced albumin synthesis. The data are as yet too meager and the results too conflicting, particularly in patients with ascites, and evidence concerning the metabolism of protein in the peritoneal cavity will be necessary before interpretations can be final. Because the presence or absence of ascites may be important in determining the rate of albumin catabolism the various factors which contribute to ascitic fluid accumulation appear to be closely linked to protein metabolism. Consequently, sodium retention, endocrine factors, portal vein pressure

and membrane permeability, all of which have been incriminated in ascites formation, may have a profound effect upon the apparent rate of albumin catabolism and synthesis. The relative importance of these factors must, therefore, be delineated before defective albumin synthesis can be declared the primary cause of fluid retention in liver disease. Tagged globulin, on the other hand, appears to have an increased rate of catabolism regardless of the presence of ascites, which strongly suggests an abnormally high gamma globulin synthesis in liver disease patients. Resolution of the problems mentioned and extension of the use of such isotope-labeled proteins may not only yield information concerning albumin and globulin metabolism but may also be of great aid in determining the therapeutic value of protein anabolism produced by dietary protein, calories, or endocrine agents.

FURTHER STUDIES ON HYDROXYPHENYLURIA

Hydroxyphenyluria occurs in premature infants fed diets high in protein and low in ascorbic acid, in scorbutic infants or adults, guinea pigs, and monkeys fed tyrosine in relatively large doses (*Nutrition Reviews* **8,** *260 (1950)*). The progress made in elucidating the nature of this defect *in vitro* has been reviewed (*Ibid.* **10,** *117, 212 (1952)*; **11,** *17 (1953)*). In interpreting these results as applied to the clinical observations, it is important to recognize the fact that agents which promote the oxidation of tyrosine *in vitro* may not always be effective when given to the whole animal.

The variability of clinical response to numerous agents administered to premature infants demonstrating hydroxyphenyluria has been reported by H. M. Nitowsky, C. D. Govan, and H. H. Gordon (*Am. J. Dis. Child.* **85,** *462 (1953)*). Folacin was administered to 20 premature infants in oral doses varying between 5 and 30 mg. and in parenteral doses of 15 to 30 mg. for variable periods of time. Five of these infants responded by a marked reduction in the excretion of hydroxyphenyl derivatives. Two of these infants responded when the folacin was given by intramuscular injection after failure of response during a period of oral administration. Those infants failing to respond to folacin responded in the expected manner to ascorbic acid or to another active agent.

Since this defect responds regularly to ascorbic acid administration, it might have been expected that the citrovorum factor would prove to be more effective than folacin. Such was not the case in these premature infants, citrovorum factor being successful only once in eleven trials. The effectiveness of vitamin B_{12} was also tested and responses were found in two of eight trials. Liver extract was similarly effective, with two responses in ten trials. Among a number of other compounds tested in this situation, dimercaprol (BAL) was effective only once in eight trials. Methylene blue and alpha-tocopherol were found to be ineffective.

S. Z. Levine, H. L. Barnett, C. W. Bierman, and H. McNamara (*Science* **113,** *311 (1951)*) found that corticotropin proved to be effective in premature infants while cortisone did not. Because of the inconsistency of response found in premature infants when several agents which sometimes reverse tyrosine-induced hydroxyphenyluria are administered, Nitowsky and co-workers have suggested that the absence of ascorbic acid necessitates the use of different metabolic pathways for the degradation of the deaminated residues of tyrosine and phenylalanine. It was also suggested that this hypothetic alternative may represent a chain reaction requiring for completion a factor or series of factors in which the absence of only

one may be responsible for the results obtained.

Further studies on the metabolism of L-tyrosine in scorbutic monkeys by R. J. Salmon and C. D. May (*J. Lab. Clin. Med.* **41,** *376 (1953)*) have confirmed their previous observations (*Ibid.* **36,** *591 (1950)*; *Arch. Biochem. Biophys.* **32,** *220 (1951)*) that both folacin and citrovorum factor are without effect in preventing the excretion of hydroxyphenyl compounds by scorbutic monkeys loaded with L-tyrosine. Since the monkey is the only species in which this phenomenon has been observed, they suggest that the degradation of tyrosine by this species may follow a different pattern. Their finding that corticotropin was without marked effect while cortisone had a variable effect suggests another possible difference between the young monkey and the premature infant. D-ascorbic acid had a small and delayed effect in reducing the tyrosyluria while L-ascorbic acid was immediately effective in the monkey. This observation would suggest that *in vitro* studies (*Nutrition Reviews* **11,** *17 (1953)*) showing an equal effect from these two compounds should be interpreted in the light of whole animal experiments.

Although the exact metabolic role of folacin, its derivatives and corticotropin is still a matter of postulation, it is becoming increasingly clear that the rate of conversion of folacin into citrovorum factor is not the limiting reaction involved in tyrosine metabolism. It has been suggested but not confirmed that corticotropin and cortisone will spare available stores of ascorbic acid and prolong the life of scorbutic guinea pigs. Hence the mechanism of action of these compounds may be different from that of the folacin group.

The interrelations in metabolism among ascorbic acid, folacin, the pituitary and adrenal hormones and vitamin B_{12} are exceedingly complex. The hydroxyphenyluria occurring in scorbutic animals fed large amounts of L-tyrosine has been one of the mechanisms studied in an effort to elucidate some aspects of this relationship.

EFFECT OF THYROID STIMULATION ON HEALTH OF DAIRY COWS

It was demonstrated experimentally a number of years ago that when lactating dairy cows are given thyroxine or iodinated casein (protamone) in moderate doses a stimulation of milk production results. This galactopoietic effect has been reported by many investigators, and several excellent reviews covering such studies are available (E. P. Reineke, *Vitamins and Hormones* **4,** *207 (1946)*; K. L. Blaxter, Reineke, E. W. Crampton, and W. E. Petersen, *J. Animal Sci.* **8,** *307 (1949)*). Most of the reported studies were conducted over a limited period, however, and as a result, little or no information is available regarding the effects of iodinated proteins on the productive life of dairy animals. In addition, little is known concerning the effects on general health and breeding efficiency of prolonged administration of such compounds.

An extensive study on these questions as well as on the effects of thyroid-stimulating substances when used in commercial practices on dairy farms, was reported from Great Britain (F. B. Leech and G. L. Bailey, *J. Agr. Sci.* **43,** *236 (1953)*).

Thirty-seven dairy herds (herd size of 30 to 50 cows) the official milk yields of which are available participated in the study. They represented the Friesian, Shorthorn, Ayrshire, Jersey and Red Poll breeds. Half of the cows in each herd were given the course of treatment while the other half served as controls or untreated animals. In all, data were collected on a total of 1172 treated and 1163 untreated cows. The calving date served as the means

of assigning animals to the "treated" or the "control" groups. The cows were listed by calving date and every other animal was assigned to treatment.

The course of treatment consisted of administering daily, per cow, for eight weeks beginning the twelfth week after calving, 4 pounds of a specially prepared dairy cube. After eight weeks of treatment, the dose was reduced at the rate of one pound each week so that treatment was discontinued after eleven weeks, or twenty-three weeks after calving. The experiment was conducted for a three-year period so that records were available on a number of cows that received the treatment through three successive calvings.

The special dairy cube contained 80 mg. of L-thyroxine per 4 pounds during the last two years of the study. During the first year it contained iodinated casein (15 to 30 g. per 4 pounds, depending upon its thyroid-stimulating potency).

Rate of disposal of the animals from the herds as a criterion of thyroxine influences on animal health failed to reveal any marked effects of treatment. The average disposal age of treated and control animals was seventy-five and four-tenths and seventy-five and five-tenths months, respectively. There was also no striking effect on the general health, based on other criteria, from thyroxine treatment, although there were differences of statistical significance. For example, 6.5 per cent of the treated cows suffered digestive disorders, whereas only 4.0 per cent of the controls were affected; 2 per cent of the treated cows had milk fever, whereas only 1 per cent of controls had this disorder. The percentage suffering from lactation tetany (hypomagnesemia) was 0.68 among thyroid-treated and 0.09 among control animals.

Thyroxine treatment did not have any consistent effect on calvings. After one season of treatment, there were a total of 56 abnormal calvings (abortions, dead calves at birth, or abnormal presentations) out of 835 total calvings among the treated animals and 86 out of 822 total calvings among controls, a highly significant reduction in abnormal calvings from thyroxine treatment. However, after two seasons of treatment the difference between treated and controls was not significant (33 abnormal out of 353 calvings for treated compared with 39 abnormal out of 368 calvings for control animals).

The length of the gestation period was unaffected by thyroxine treatment. Likewise, there was no indication that treatment had any influence on the efficiency of coitus or interval between parturitions.

During the period of treatment there was a marked response in daily milk yield, but the fat content of the milk was not raised. However, this response was obtained only in older cows (third lactation or later). The net result of thyroxine use during the first and second lactations was a depression in total milk yield.

Treatment with thyroxine had the general effect of shortening the length of the lactation period. This effect was most marked in cows that received the thyroxine in the spring and was absent in cows that received treatment in the winter. These results seem to demonstrate that thyroidal stimulation in first and second lactations in dairy cows is clearly unprofitable. The response in the form of increased milk yield in older cows, amounting to about 3 per cent of the lactation total, was obtained without any noticeable detrimental influence on the health of the animals.

BACTERIAL CULTURES IN THE NUTRITION OF POULTRY

Nutritionists recognize the contributions which intestinal bacteria make to the vitamin requirements of the host animal. There is considerable variation between

species in the quantities of various vitamins made available to the host through bacterial synthesis. This is due in part to the fact that in animals with a single stomach compartment much of the bacterial activity occurs in the cecum and the vitamins which are synthesized are poorly absorbed. Ruminants appear to be able to obtain adequate quantities of all the B-vitamins as a result of synthesis by bacteria in the alimentary tract. The data regarding this phenomenon in man are limited; however, it has been suggested that prolonged antibiotic therapy may produce signs of vitamin deficiency as a result of alteration of the bacterial flora (H. M. Gewin and G. J. Friou, *Yale J. Biol. Med.* **23,** *332 (1951)*). Also, it has been observed that the oral administration of streptomycin to adult men results in a reduced urinary excretion of biotin and of niacin metabolites (H. P. Sarett, *J. Nutrition* **47,** *275 (1952)*).

The observation that the feeding of antibiotics would increase the growth of animals under certain experimental conditions (*Nutrition Reviews* **8,** *298 (1950)*) would appear at first glance to be contradictory to these considerations, because the most significant growth response occurs when the antibiotics are added to diets containing limiting amounts of various B-vitamins (*Ibid.* **10,** *107 (1952)*; K. Guggenheim, S. Halevy, I. Hartmann, and R. Zamir, *J. Nutrition* **50,** *245 (1953)*). It has been suggested that the antibiotics improve growth by so altering the intestinal flora that there is an increase in the numbers of bacterial forms which synthesize vitamins required by the host or a decrease in those forms which utilize these vitamins and thus compete with the host.

The recent reports of G. W. Anderson, S. J. Slinger, and W. F. Pepper (*J. Nutrition* **50,** *35 (1953)*), and of Anderson, Slinger, Pepper, and M. U. Hauser (*Ibid.* **50,** *47 (1953)*), are of interest in view of the above considerations. These workers studied the effects of feeding bacterial cultures with and without antibiotics to chicks and turkey poults. Observations were made on growth rate and efficiency of gain, and on the pH and bacterial count of cecal contents.

In the chick experiments Columbian Rock male chicks were fed a basal diet containing 60.75 per cent ground yellow corn, 33 per cent soybean oil meal, 2 per cent dehydrated cereal grass, 0.5 per cent ground limestone, 3.25 per cent steamed bone meal, 0.25 per cent iodized salt and 0.25 per cent fish oil (300 I.C.U. of vitamin D and 1500 I.U. of vitamin A per gram). The following supplements were added per 100 pounds of diet: riboflavin, 150 mg.; niacin, 900 mg.; calcium pantothenate, 300 mg.; vitamin B_{12}, 0.2 mg.; choline chloride (25 per cent), 70 g.; potassium iodide (90 per cent), 150 mg.; and manganese sulfate (technical), 10 g. Groups of chicks were fed this basal diet, or this diet supplemented with various bacterial cultures with and without 10 parts per million (p.p.m.) of procaine penicillin G. The bacterial cultures which were fed were isolated from the cecal contents of chicks and were maintained in nutrient broth, thioglycollate, or skim milk mediums. Ten ml. of the liquid culture were added per 100 g. of basal diet. The controls received uninoculated mediums. The bacteria used were two strains of atypical *Escherichia coli*, *E. coli*, micrococci, and anaerobes. The various diets and water were given ad libitum to chicks for a five-week experimental period.

The feeding of atypical *E. coli* 2 increased the rate of growth of chicks both in the presence and absence of dietary penicillin. Atypical *E. coli* 1 and *E. coli* increased growth only in the presence of penicillin. The feeding of micrococci depressed growth in the absence of penicillin, but increased growth in the presence of penicillin. The feeding of anaerobes did not affect growth either in the presence or absence of penicillin. The feeding of penicillin increased growth except when added to the basal diet supplemented with uninoculated skim milk mediums. Penicillin enhanced the growth response to atypical *E. coli* 1 and *E. coli*,

but did not affect the response to atypical *E. coli* 2. After the five week observation periods the chicks were killed and the cecal contents were collected for pH determinations and bacteriologic study. The number of aerobes, coliforms, lactobacilli and enterococci in the cecal contents was determined. The results on these studies indicated that the feeding of penicillin resulted in a reduction in the pH of cecal contents and a general decrease in the numbers of all microorganisms studied.

In a second experiment with chicks an effort was made to determine whether the growth response observed as a result of feeding the cultures of *E. coli* was due to the cells or to a metabolite produced by the cells and secreted into the mediums. A mixed culture containing the *E. coli* and the two strains of atypical *E. coli* was used. The basal diet used previously was supplemented with the mixed culture, with a cell-free filtrate of the culture (obtained by centrifugation), or by washed cells which were resuspended in sterile water. These preparations were added to the basal diet both in the presence and absence of penicillin. The growth data obtained in this experiment were not clear-cut. The data presented suggest that the growth-promoting activity of the mixed culture was due both to the cells themselves and to a factor or factors found in the filtrate. The bacteriologic studies and pH determinations on cecal contents in this experiment gave essentially the same results as wereb otained in the previous experiment.

Similar experiments were conducted using Broad Breasted Bronze poults. The basal diet and general plan of experiment were similar to those used in the chick studies. Mixed coli and *E. coli* cultures were used, and in some experiments the cells were killed by boiling before being added to the diet. The poults were fed for four weeks. The growth-promoting effect of penicillin was again observed. This effect was greatest when penicillin was added to the diet containing the culture of mixed coli. The addition of the culture of mixed coli had no effect on growth in the absence of penicillin, but increased growth in the presence of penicillin. *E. coli* feeding increased growth in both the presence and absence of penicillin. Neither the filtrate from the mixed coli culture nor the killed mixed coli cells improved growth either in the presence or absence of penicillin. The effects of the various supplements on bacterial counts and pH of cecal contents were quite similar to the results obtained in the chick experiments.

In the final experiment a study was made of the growth-promoting effect of atypical *E. coli* type 2 on poults both in the presence and absence of dietary penicillin. Also, since coliform bacteria produce lactic acid, the effect of feeding 1 per cent lactic acid was studied. The feeding of either the bacterial culture or penicillin improved growth. The combination of the two was no more effective than penicillin alone. Lactic acid feeding did not significantly affect growth either in the presence or absence of penicillin.

These studies show that it is possible to stimulate the growth of poultry by feeding bacterial cultures originally obtained from cecal contents of birds. This is another demonstration of the importance of bacteria in the alimentary tract for the nutrition of the host. The data presented also indicate an interrelationship between the bacterial cultures and penicillin in promoting growth. This is additional evidence that at least part of the growth-promoting effects of antibiotics may be related to an effect on intestinal flora.

COPPER AND IRON METABOLISM

That copper is needed along with iron for the formation of hemoglobin is now well known. It was shown by C. A. Elvehjem and E. B. Hart (*J. Biol. Chem.* **95,** *363*

(*1932*)) that in the pig, copper deficiency, even in the presence of iron, resulted in hypochromic, usually microcytic, anemia as well as in debility. The mechanism of the interrelation between copper and iron metabolism, however, remains unknown. A series of publications by Wintrobe and his collaborators, reporting on a thorough comparison of copper and iron deficiencies in the pig and examining in detail the effect of copper deficiency on iron absorption from the intestine in the rat, now establishes at least one element in the elucidation of this problem.

In one experimental series, M. E. Lahey and co-workers (*Blood* **7,** *1053* (*1952*)) fed a total of 70 swine a basal diet consisting of evaporated cow's milk diluted with tap water. One group received supplements of iron, another group supplements of copper, a third group received both copper and iron, leaving a fourth group of 4 animals unsupplemented with either metal. It was found that a deficiency in copper alone decreased growth rates only slightly and did not affect the appearance of the animals, which kept a sleek coat with excellent texture. Only when severe anemia developed did the animals show definite deficiency symptoms, in particular a listless attitude, decreased appetite, and, in some animals, marked bowing of the legs. Serum calcium, phosphorus and alkaline phosphatase determinations were conducted in only one animal; normal values were found.

Marked anemia was observed in all animals on copper-deficient diets after a lag of about forty days. In accordance with previous reports, this anemia was microcytic and hypochromic. Normoblastic hyperplasia of the bone marrow was observed. Also present were hypoferremia and increased iron-binding capacity of the plasma. Essentially similar symptoms were observed in the iron-deficient animals. The most notable differences were related not to the erythrocytes, but to the leukocytes and platelets, namely, leukopenia was observed in copper deficiency and thrombocytosis in iron deficiency.

C. J. Gubler and co-workers (*Blood* **7,** *1075* (*1952*)), in another experimental series, found normal catalase activity in erythrocytes, liver and kidney of copper-deficient swine. The mature erythrocytes showed normal amounts of free protoporphyrin. Serum bilirubin levels, reticulocyte counts and osmotic fragility of red blood cells also remained normal, suggesting that erythrocyte destruction was not accelerated. Red cell copper content appeared relatively stable on a copper-deficient diet for at least two weeks. During this period plasma levels fell to scarcely detectable values. By contrast, in iron deficiency a slight elevation in the plasma copper level is noted. Copper deficiency also appeared to be characterized by impaired ability to absorb iron from the gastrointestinal tract, incomplete mobilization of iron from the tissues and inability to utilize parenterally administered iron for hemoglobin synthesis, even when this iron was presented to the bone marrow in normal quantities. A combination of copper and iron deficiencies accelerated the occurrence of all the manifestations noted in copper deficiency alone.

In order to study in more detail the apparent impairment in the absorption of iron from the intestine in copper deficiency, M. S. Chase, C. J. Gubler, G. E. Cartwright, and M. M. Wintrobe (*J. Biol. Chem.* **199,** *757* (*1952*)) turned to the use of rats as experimental animals. These workers used as basal diet a milk ration supplemented with iron which was spectroscopically free of copper. In one experiment a control group was left unsupplemented. Four experimental groups received copper supplements of 0.05 mg., 0.25 mg., 0.50 mg. and 1.0 mg. per rat per day. After a period of twenty-eight days, radio-iron was substituted for inert iron. Fourteen days later, the mineral supplements were discontinued and four days subsequent to this the animals were killed and total body radio-iron,

inert iron and copper were determined. Radio-iron incorporation was taken as a measure of iron absorption. It was found that increasing the concentration of copper in the diet from 0 to 0.50 mg. per rat per day increased the retention of iron in the body. With respect to hemoglobin concentration the control group was significantly lower than the copper-supplemented groups, but no differences were observed among the latter.

In a second experiment designed to determine whether the role of copper in iron absorption was dependent on its being administered simultaneously with the iron, a single dose of iron was administered together with increasing doses of copper to five groups of animals fed the basal diet supplemented with 0.5 mg. of iron and 0.05 mg. of copper per rat per day. In this case, no significant difference in the amount of radio-iron absorbed was noted among the five groups. To confirm the fact that copper concentrations in tissues rather than the level of copper in the diet influenced iron absorption, radio-iron was next administered to five groups of animals. All had received for thirty-eight days the basal diet supplemented with iron. Four groups had also received various daily dosages of copper, but the supplements had been omitted forty-eight hours before radio-iron administration. Significant differences in iron absorption were found to correspond to increasing tissue copper levels up to a maximum corresponding to a previous intake of 0.25 mg. of copper per rat per day.

These observations make it clear that copper influences the absorption of iron in the rat. Failure of previous investigators to demonstrate this effect is probably due to exclusive concentration on the study of hemoglobin levels, or to the fact that chemical procedures were less sensitive than measurement of radio-isotope concentrations. It also appears from the experiments of Chase and his collaborators that copper concentrations in the tissues, rather than copper levels in the diet, are the paramount factors in this relationship. The authors caution against extrapolating from these results the conclusion that the only role of copper in mammalian tissues is in relation to iron absorption, or that copper deficiency anemia is simply the result of failure to absorb adequate amounts of iron. On the other hand, they point out that increasing the amount of copper in the diet did not favor greater iron absorption above a maximum corresponding to the administration of 0.25 to 0.50 mg. per day, which strongly argues against the possibility suggested by some authors that exposure to high concentrations of dietary copper would in itself result in the production of hemochromatosis.

EFFECT OF VITAMIN B_{12} ON WOUND HEALING

The importance of nutrition in wound healing has long been recognized. The available data suggest that many different nutrients affect the healing of wounds. In particular, acute and chronic protein deficiency leads to a delay in wound healing, serious wound infection, and increased morbidity and mortality. Since it appears that under certain circumstances vitamin B_{12} may participate in protein synthesis and act as a growth factor, C. W. Findlay (*Proc. Soc. Exp. Biol. Med.* **82,** *492 (1953)*) has recently investigated the possible role of vitamin B_{12} in wound healing in normal and protein-depleted rats.

Sherman rats (sex not stated) previously fed a commercial chow ad libitum were given one of three experimental diets for three weeks preceding and for six days following experimental wounding. Two groups were offered diets containing about 500 calories per 100 g. of food. The calories supplied by protein were about 15 per cent of the total for one group and only 1 per

cent for the other group. A third group of rats were kept on the commercial chow which supplied 320 calories per 100 g. of diet, 30 per cent of the calories coming from protein. The various diets were offered ad libitum and the daily intakes were measured. No attempt was made to maintain a constant intake, nor was the pair-feeding technic utilized. Comparable numbers of animals, about 8 in each group, were given daily intramuscular injections of isotonic saline or an equivalent amount of saline containing 0.5 micrograms of vitamin B_{12} per 100 g. body weight. These solutions were given either throughout the experimental period or only in the postoperative period.

The technic of wounding is not described, but presumably the wounds were of the types utilized and described by Findlay in a previous publication (C. W. Findlay and E. L. Howes, *Surgery* **28,** *970 (1950)*). "A midline abdominal incision 5 cm. in length was made under ether anesthesia. It was deepened to the linea alba, but the fascia was not incised. The incision was closed with interrupted sutures of No. 30 steel wire. In some animals the peritoneal cavity was entered through a muscle-splitting rectus incision and then the wound was closed in layers with the same material."

The rats were killed on the sixth postoperative day, the presence or absence of gross wound infection was noted, and the tensile strength of three strips 1 cm. wide of each noninfected wound was measured. The results of the measurements for each animal were averaged and recorded as the average tensile strength per centimeter of wound. Sections were also taken for microscopic examination. The technics of tissue fixation and staining are not reported.

Preoperatively, the animals on the low protein diet lost weight, whereas those on the higher protein diets gained weight. The average daily food intake of the low protein group during the preoperative period was 12.3 g., while that of the higher protein groups was 16.2 g. The average gain in weight of the latter during the preoperative period was about 12 per cent, the average loss in the former about 15 per cent. Postoperatively, almost all animals in the three groups lost weight, about 2 per cent on the average. The average daily food intake during the postoperative period of the low protein animals dropped to 9.6 g., that of the higher protein groups to 12.2 g. No effect of supplementary vitamin B_{12} on body weight or food intake was noted in any group.

The average tensile strengths of the wounds in the two groups of animals on the higher protein diets were significantly higher than those of the low protein diet group. Histologically, the wounds of the latter showed diminished fibroplasia, derangement of the normal polarity of the fibroblasts, and evidence of tissue edema. Grossly, there was also a higher incidence of wound infection among the low protein animals.

Supplementary vitamin B_{12} had no apparent influence on the incidence or severity of wound infection, tensile strength, or histologic appearance of the wounds among the animals on low protein diets, but the wounds of the animals on the higher protein diets which were supplemented with vitamin B_{12} were significantly stronger than those of the animals which received only saline. No differences were observed among those animals given the vitamin B_{12} both preoperatively and postoperatively and those given vitamin B_{12} postoperatively only.

In a subsequent series of experiments, rats fed either of the higher protein diets with or without supplementary vitamin B_{12} given postoperatively were killed on days 3, 4, 5, 6, 7 and 8 after operation. On the third postoperative day, the average tensile strength of the wounds was significantly higher in the animals receiving vitamin B_{12} than in those receiving only saline. However, only 3 animals were tested in each group. On subsequent days there was no significant difference in the average change of tensile strength from the second to the eighth day between the two groups,

nor was there a significant difference in the tensile strength of the wounds at the eighth postoperative day. It is not clear from the data presented (averages only) whether there were significant differences on days 4, 5, 6 and 7.

These preliminary experiments suggest that vitamin B_{12} may play a role in the early period of wound healing in the rat. However, many additional data (for example, the course of healing in vitamin B_{12}-deficient animals) are required to establish the mode of action and significance of vitamin B_{12} in wound healing.

GASTRIC SURGERY AND FAT ABSORPTION

The difficulty with which normal nutrition is maintained in patients who have undergone removal of all or large parts of the stomach is well recognized. Whether this problem is due to inability to eat normal amounts of food or to lack of normal absorption is not always easily determined in a given patient, and in those patients whose absorption is deficient the possible causes of this defect have not been delineated. Mechanical, secretory, or enzymatic defects, or a combination of these may be at fault, and attempts to correct or prevent such defects by the proper surgical procedures have been the subject of investigation in both animal and man. An excellent review of this problem and studies of it have been presented by T. C. Everson (*Internat. Abstr. Surg.* **95,** *209 (1952)*).

Everson has analyzed the data available from his own studies and from the work of others in regard to total gastrectomy, partial gastrectomy, type of anastomosis and medications, chiefly in regard to their effects upon fat and nitrogen absorption, but also including data upon other nutrients, in both man and animals. With due regard for the possible fallacy in this type of compilation of material he concludes that there is evidence of impaired fat assimilation in nearly two thirds of patients with total gastrectomy and similar impairment in a higher percentage of dogs undergoing this operation. Likewise, nitrogen absorption is defective in approximately one third of the patients and in over half of the dogs so studied.

Following partial resection of the stomach there appears to be fat and nitrogen absorptive difficulty in nearly the same percentage of patients as was found in the total gastrectomy group. The relative severity of these defects in the two sets of patients was not analyzed, however, and the difficulties of drawing conclusions from a review of the literature are emphasized by the author in his notation that many of the patients in the subtotal gastrectomy series were specifically selected for study by various authors because of the presence of digestive complaints. Thus the nutritional deficiencies in subtotal gastrectomy are probably, as might be expected, less frequent than those after total resections.

In the opinion of most authors, the use of an esophagoduodenal anastomosis was found to be preferable to an esophagojejunal anastomosis which to some degree by-passes the normal secretory mechanism of the biliary system and pancreas. The author's personal studies in dogs have confirmed that better nitrogen absorption occurs with the former anastomosis after total gastrectomy, but he was unable to note any significant advantage over the latter type of anastomosis in regard to fat absorption.

Among numerous medications tried by various workers in attempts to improve absorption, only pancreatin appeared to have significant merit, while various anticholinergic agents used alone did not regularly affect fat or nitrogen loss in the feces of

patients or dogs. This was true in both total and partial gastrectomy.

The surgical production of reservoirs or pouches to replace the stomach has been attempted by numerous workers, but the success of such procedures is not yet established. Similarly, evidence that absorption improves with the passage of time after operation is not available. The use of a schedule of frequent feedings after gastrectomy has been common, but in spite of excellent clinical reasons for such a regimen the author points out that only in a very few animals has frequent feeding been demonstrated to improve fat absorption, while in the few human subjects on whom data are given the improvement reported is probably insignificant. Further investigation is needed in regard to this procedure. Likewise, the efficacy of "Tween 80," bile substitutes and other medications remains unproven and in need of study.

In view of the findings reported by Everson in his review a more recent study of the problem of fat absorption after gastric surgery in dogs is worth noting (R. B. Welbourn, G. A. Hallenbeck, and J. L. Bollman, *Gastroenterology* **23,** *441 (1953)*). These workers undertook a comparison of fecal fat loss in dogs with varying degrees of resection and with differing anastomoses. Mean fat loss in 7 control dogs was 2.7 per cent while that in 6 dogs with partial resection and a gastroduodenostomy was 3.7 per cent. In 6 dogs with partial resection and gastrojejunostomy mean fat loss was 5.2 per cent. These differences were found to be statistically significant.

Total gastrectomy with esophagoduodenostomy or with esophagojejunostomy, 5 dogs in each group, yielded marked increases in fecal fat, the mean being 13.5 per cent in the former group and 13.7 per cent in the latter. Individual variation in steatorrhea was quite wide in these animals and repetition of the tests at ten weeks after operation, approximately six weeks after the original determinations, revealed a significant improvement in the amount of fat absorbed, without complete correction of the steatorrhea. Simple gastroenterostomy without resection yielded no change in 2 dogs, but a loss of 26 per cent of fat intake occurred in a third. The latter animal returned to normal after reoperation and restoration of the whole normal gastrointestinal pathway. Vagotomy alone in 4 dogs was without effect on fat absorption, while vagotomy after previous partial gastrectomy increased fecal fat in one of 2 dogs studied.

The nutritional difficulties experienced in these animals were reflected in the weight changes which occurred. Special feeding care with offerings of extra food prevented loss or even allowed some gain in weight after operation in 7 of 8 dogs with partial gastrectomy and in 7 of the 10 dogs which had total gastrectomy. Cessation of this care, however, resulted in weight loss in all the dogs except one with partial gastrectomy, although they received diets sufficient to maintain intact animals. The resulting emaciation was found to be irreversible if allowed to progress too far. No effect on the weight was observed in those animals undergoing gastroenterostomy or vagotomy alone.

In 3 dogs, one with partial gastrectomy and gastrojejunostomy, a second with total gastrectomy and esophagoduodenostomy, and a third with total gastrectomy and esophagojejunostomy, the effect of increasing the fat intake upon the fecal fat excretion was determined. Increases of from 50 to 500 g. per day in fat intake resulted in no increase in the percentage loss of fat in these animals.

Although the studies of Welbourn and his associates reveal relatively small degrees of steatorrhea in animals undergoing removal of part or all of the stomach, it is apparent that increased loss of nutrients is a definite sequela of such surgery. It is also clear that in dogs, at least, the type of anastomosis performed was without effect on the degree

of fat loss. These results are in essential accord with those reviewed by Everson (*loc. cit.*), and serve to emphasize the nutritional difficulties which may be incurred in patients undergoing similar surgery. Certain differences in the situation in the animals and in the conditions existing in man should be noted, however. The fat intake in these dogs was approximately 8 g. per kilogram of body weight, representing a proportion of fat in the diet which is seldom if ever attained in the human subject. Also, these animals are usually operated on without previous illness such as may exist in man and are not subject to the hazard of recurrent or inoperable malignant tissue. It is thus apparent that findings in animals cannot be strictly transferred to the clinic. Nevertheless, the accumulation of this type of information should serve as a stimulus to surgeons interested in attempting to obtain better physiologic results in gastric surgery.

In both reports the possible physiologic mechanisms responsible for steatorrhea as well as the other phenomena of malabsorption after gastrectomy are discussed. Although no one defect is readily apparent, both sets of authors emphasize the therapeutically important point that the nutritional problem is lessened or eliminated if increased food intake is attained.

VITAMIN AND CALORIC DEFICIENCIES AS RELATED TO COLD STRESS

Resistance of animals to cold is significantly impaired under certain nutritional deficiency conditions, according to B. H. Ershoff (*Proc. Soc. Exp. Biol. Med.* **78,** *385 (1951)*; **79,** *559, 580 (1952)*). In these publications it was reported that rats deficient in pyridoxine, riboflavin, or vitamin A were less resistant to cold stress than rats receiving the same diets supplemented with these vitamins. L. P. Dugal and M. Thérien (*Canad. J. Res., Section E* **25,** *111 (1947)*) had shown earlier that guinea pigs deficient in ascorbic acid had an impairment of resistance to cold stress.

In a recent study, Ershoff has investigated the influence of pantothenic acid deficiency and of caloric restriction (a condition which often must be considered in vitamin deficiency studies) on the resistance of rats to cold stress (B. H. Ershoff, *J. Nutrition* **49,** *373 (1953)*). The basal ration used consisted of the following in per cent: sucrose, 61; casein, 24; salt mixture, 5; cottonseed oil, 8; and wheat germ oil, 2. Each kilogram of the ration was supplemented with the following, in milligrams: thiamine hydrochloride, 10; riboflavin, 10; pyridoxine hydrochloride, 10; niacin, 60; biotin, 5; 2 methyl-naphthaquinone, 5; folacin, 10; ascorbic acid, 100; para-aminobenzoic acid, 400; inositol, 800; vitamin B_{12}, 0.1; and choline chloride, 2000. In addition, 6000 USP units of vitamin A and 600 USP units of vitamin D were added per kilogram of ration. Control animals received the same ration supplemented with 60 mg. of calcium pantothenate per kilogram of diet.

Eighty weanling rats were used in the study, which consisted of three experimental groups, namely: Group A (40 rats), basal diet (pantothenic acid-deficient) fed ad libitum; group B (20 rats), basal diet supplemented with calcium pantothenate and fed ad libitum; group C (20 rats), ration of group B fed at a level equivalent to the rate of consumption in group A.

Groups A and C consumed an average of 8.7 g. of ration daily and gained 79 and 112 g. per rat, respectively, in twenty-eight days. This indicated that pantothenic acid deficiency limits growth beyond that accountable by impairment of food consumption. Rats in group B consumed an average of 12.4 g. of ration per rat daily

and gained 144 g. of body weight in twenty-eight days. At the end of four weeks, of the 38 surviving animals in group A 31 were considered depleted of pantothenic acid, as judged by stationary or declining body weights. At this time the rats of group C (restricted-fed) had reached a plateau in body weight.

Nineteen of the rats in group A and 10 of those in each of groups B and C were then placed in a refrigerator at a temperature of 2° C. The remainder of the animals were kept in the normal environment. Twenty-four hours later, the remaining 10 rats of group C (restricted-fed) were also placed in the cold environment. All rats of group B (normal ration fed ad libitum) survived twenty-eight days in the cold environment and each consumed an average of 19.7 g. of ration per day. Those in the same group kept at room temperature each consumed an average of 12.2 g. of ration daily. All the pantothenic acid-deficient rats (group A) that were subjected to cold temperature died in the first seventy-two hours of exposure. Under room conditions, however, 9 of the 12 pantothenic acid-deficient rats were still alive after twenty-eight days.

Since the pantothenic acid-deficient rats did not survive cold sufficiently to permit paired-feeding with animals on the normal diet (group C) the latter were divided so that 10 received full feedings (average of 20 g. each daily) while the other 10 received 60 per cent of full-feeding level (12 g. per day). All rats receiving full-feeding survived the cold, whereas 60 per cent of those restricted to 12 g. of feed daily died in an average of nineteen days. These findings indicated that pantothenic acid deficiency reduced the resistance of rats to cold stress, but that part of this effect was associated with a reduced resistance due to caloric restriction (reduced feed intake).

Another experiment was conducted by Ershoff to investigate further the influences of caloric restriction. It was shown that the length of survival under cold environmental conditions was dependent upon the level of caloric consumption during cold exposure as well as upon the level of caloric intake before exposure to cold. Animals that were full-fed before exposure to cold and restricted to 12 g. of feed daily per rat during exposure had a survival incidence of 30 per cent and an average survival period among decedents of eighteen days. Similar animals limited to 8 and 4 g. of feed daily all died in an average of ten and eight days, respectively.

Rats that were restricted in caloric intake to approximately 50 per cent of normal (6 g. per rat daily) during the pretest period, and fed 12 g. daily during exposure to cold, had a survival incidence of 40 per cent, with an average survival period of seventeen days for decedents. Similar rats limited to 6 g. of feed daily during exposure to cold all died in an average of three days. Rats that were restricted to 4 g. of feed each day during the pretest period all died within twenty-four hours after exposure to cold, regardless of the level of caloric intake after exposure.

Further experiments were conducted by Ershoff to determine the effects of hormones on the resistance of pantothenic acid-deficient rats to cold stress. The possibility of adrenocortical insufficiency as a contributing factor toward lowered resistance to cold was suggested by the recent observation that pantothenic acid deficiency imposes a continuous stress on the adrenal cortex (*Nutrition Reviews* **8,** *25 (1950)*; **10,** *221 (1952)*). None of the hormones tried was effective in prolonging the length of survival of pantothenic acid-deficient rats under exposure to cold. Anterior pituitary extract, growth hormone, ACTH, thyroxine, cortisone acetate, and combinations of these were without effect.

The accumulating information on the influence of vitamin deficiencies on resistance

to cold environmental temperatures is of interest and lends support to the importance of adequate nutrition under stress conditions. One important aspect of this type of study must, however, not be overlooked, and that is the lack of calories usually consumed on such diets. When such studies are conducted, it might be important to gather data simultaneously on the influence of forced-feeding so that the contributing influences of caloric restriction would be ruled out.

INFLUENCE OF CHOLINE AND PROTEIN DEFICIENCY ON THE LIVER

The question of whether or not choline is of therapeutic value in liver damage induced by protein deficiency has received considerable attention in recent years. D. Koch-Weser, J. de la Huerga, and H. Popper (*J. Nutrition* **49,** *443 (1953)*) have investigated liver damage in rats resulting from diets low in protein or in choline and have studied the influence of therapeutic doses of choline in both conditions. Their study utilized both histologic and chemical criteria to determine the therapeutic effects.

The low choline diet used by these investigators contained 20 per cent of peanut meal (alcohol extracted) and 6 per cent of casein as protein sources, 3 per cent of corn oil, 2 per cent of cod liver oil, 65 per cent of dextrose, and was supplemented with the following vitamins (milligrams per kilogram of diet): thiamine hydrochloride, 10; pyridoxine hydrochloride, 10; calcium pantothenate, 40; riboflavin, 20; niacin, 40; biotin, 0.01; and oleum percomorphum, USP, 100.

The low protein diet had the following composition (expressed in per cent): casein, 6; hydrogenated cottonseed oil, 38; cod liver oil, 5; and sucrose, 49. It was supplemented with the same vitamins as were added to the low choline diet except that 1.2 g. of choline chloride per kilogram of diet were also included.

A total of 83 rats was used in the study. The animals weighed 175 to 228 g. at the start of the experiment. The animals that were fed the low choline diet for four weeks lost an average of 15 per cent of their original body weight. Their livers were yellow and fatty and histologically were found to represent diffuse fatty metamorphosis (infiltration). No necrosis was observed. Serum esterase and alkaline phosphatase activity and hepatic activity for these enzymes were found to be unchanged from activities noted in animals fed a well-balanced laboratory diet. Bromsulfalein retention and serum bilirubin values were found to be increased in the animals receiving the low choline diet.

Rats receiving the low protein diet for four weeks lost 28 per cent of their body weight. The livers were large and brownish-yellow, and their surfaces were somewhat mottled. Numerous focal necrotic areas were detected in the liver tissue that was examined microscopically. The increased bromsulfalein retention and serum bilirubin were comparable to those observed in the choline-deficient animals. In addition, these animals on the protein-deficient diet exhibited elevated serum and hepatic alkaline phosphatase activities and lowered esterase activities in these tissues.

The remainder of the animals, after four weeks on the choline-deficient diet, were given 30 mg. of choline chloride by stomach tube in two doses sixteen hours apart. The remaining animals that had been on the low protein diet were similarly treated.

Forty hours after choline dosage, the animals previously on the low choline diet had almost completely returned to normal with respect to bromsulfalein retention and serum bilirubin concentration. The hepatic fatty changes had likewise almost com-

pletely subsided. Similarly, choline therapy in the animals fed the low protein diet caused them to improve considerably with respect to liver fat levels, bromsulfalein retention, and serum bilirubin levels. However, the elevated serum and hepatic alkaline phosphatase activities and the lowered serum esterase of these tissues were not influenced by choline therapy in these animals.

The authors consider that serum and hepatic phosphatase and esterase disturbances are evidences of liver cell damage, whereas the changes in bromsulfalein retention and serum bilirubin run parallel with fatty metamorphosis of the liver.

The conclusion that choline is without influence on liver cell damage induced by a protein deficiency is not unexpected. However, it is questionable whether the liver lesions noted by Koch-Weser and associates should be considered as evidence of damage resulting from protein deficiency. Neither the protein-deficient nor the choline-deficient diets used by these workers contained a supplement of tocopherols. This nutrient, when omitted from diets low in protein, produces not only liver necrosis but also massive lung hemorrhage (E. L. Hove, *see Nutrition Reviews* **11,** *31 (1953)*). Furthermore, the high level of fat in the low protein diet employed by Koch-Weser and associates would tend to increase the requirement for tocopherols. The low protein diet employed by these workers would therefore not seem to be an altogether appropriate one for use in attempting to evaluate choline as a therapeutic agent in liver lesions induced by protein deficiency. A further discrepancy with respect to dietary adequacy should be mentioned: neither of the diets used contained a mineral supplement.

The results of these studies indicate that choline therapy does not relieve liver cell injury arising from feeding a high fat diet deficient in protein, tocopherols, minerals, vitamin B_{12} and folacin.

EFFECT OF PLANT STEROLS ON PLASMA AND LIVER CHOLESTEROL IN THE CHICK

Investigations with several animals (rats, mice, rabbits, and dogs) have shown that plant sterols are inert dietary constituents and that little, if any, of these substances is absorbed from the gastrointestinal tract. Cholesterol, the typical animal sterol, is absorbed, although poorly, by most species if it is consumed along with an abundance of triglycerides.

Even though the plant sterols appear to be inert dietary constituents, they nevertheless possess the ability to influence the level of cholesterol in the blood plasma and liver of animals subjected to high cholesterol intake (D. W. Peterson, *Proc. Soc. Exp. Biol. Med.* **78,** *143 (1951)*). The addition of 1.3 per cent of mixed soybean sterols (95 per cent sterol) to a high (1 per cent) cholesterol diet eliminated the hypercholesteremic response of chicks that was present when the same diet was fed without the soybean sterols (Peterson, C. W. Nichols, and E. A. Shneour, *J. Nutrition* **47,** *57 (1952)*).

As a possible explanation for these results, Peterson and his associates at the University of California offered the suggestion that the plant sterols may interfere with cholesterol absorption from the gastrointestinal tract. A somewhat similar explanation was offered by W. M. Sperry and W. Bergmann (*J. Biol. Chem.* **119,** *171 (1937)*), who noted that sitosterols, a complex mixture of sterols occurring in the oils of the higher plants, prevented the accumulation of cholesterol in the livers of mice.

In a recent paper, the California workers have reported further results on the rela-

tionship between animal and plant sterols in the nutrition of chicks (D. W. Peterson, E. A. Shneour, N. F. Peek, and H. W. Gaffey, *J. Nutrition* **50,** *191 (1953)*). These workers used a natural diet containing 0.05 per cent cholesterol and 0.02 per cent of other sterols. The diet contained 4.5 per cent of cottonseed oil in addition to 3 per cent of crude fat in the remaining ingredients.

When the basal diet was fed to 2 to 3 week old chicks for two to five weeks, the plasma total cholesterol ranged between 80 and 110 mg. per 100 ml. The same diet containing 1 per cent of cholesterol as a replacement for an equal amount of ground barley produced an average plasma cholesterol level of 610 mg. per 100 ml. in two weeks and 828 mg. per 100 ml. in five weeks. The omission of the cottonseed oil from the diet containing 1 per cent of cholesterol resulted in plasma cholesterol values of 156 mg. per 100 ml. of blood.

Feeding the basal diet (cottonseed oil included) and 1 per cent of cholesterol plus 1 per cent of soybean sterols to the chicks resulted in plasma cholesterol values ranging between 150 and 190 mg. per 100 ml. Substituting 0.5 per cent of soybean lecithin (reported to contain 65 per cent phospholipids) for the soybean sterols resulted in severe hypercholesteremia (860 to 1006 mg. per 100 ml. of blood) which could again be prevented by the further addition of 1 per cent of soybean sterols. This diet resulted in plasma cholesterol values of 148 mg. and 228 mg. per 100 ml. at two and five weeks, respectively.

The level of dietary soybean sterols needed to overcome the hypercholesteremia resulting from a 1 per cent dietary level of cholesterol was also investigated. Dietary soybean sterol levels of 0, 0.25, 0.50, 1.0, 2.0, and 3.0 per cent resulted in plasma cholesterol levels of 752, 481, 229, 153, 100, and 95 mg. per 100 ml., respectively, in a two-week feeding trial. Essentially similar, although slightly higher, values were obtained when the feeding period was extended to six weeks. Total cholesterol levels in the liver essentially paralleled the blood levels. For example, after six weeks the basal diet resulted in liver cholesterol levels of 1 per cent (dry weight basis). One per cent of dietary cholesterol increased this to 6 per cent, whereas the further dietary supplement of 1 and 2 per cent soybean sterols lowered the liver cholesterol values to 2.4 and 1.4 per cent, respectively.

Sitosterols, when used as a 1 per cent supplement to the diet containing 1 per cent of cholesterol, reduced the plasma sterol levels from 892 mg. to 160 mg. per 100 ml. in a four-week feeding trial. Beta-sitosterol, ergosterol, and stigmasterol were likewise effective in correcting the hypercholesteremia in the chick induced by feeding the diet containing 1 per cent of cholesterol.

The sterols tested by Peterson and associates, which were found to prevent hypercholesteremia in the chick, differ only slightly in structure from cholesterol itself. The possibility was therefore suggested that their effects may be explained on the basis of inhibition of an enzyme system concerned with cholesterol absorption and possibly involving cholesterol esterification. The soy sterols were esterified, eliminating the free hydroxyl group in the 3-position (caprate esters). These substances no longer possessed the ability to control hypercholesteremia in the chick induced by dietary cholesterol. It was further noted that elimination of the free hydroxyl group in the 3-position of the cholesterol (cholesteryl caprate) almost completely eliminated its ability to produce hypercholesteremia in the chick.

These observations suggest that the ability of plant sterols to influence hypercholesteremia in the chick may be through their behavior as analogues in enzyme systems concerned with the esterification of cholesterol during its absorption.

EVALUATION OF PROTEIN QUALITY

Despite a half century of controversy, new methods for the evaluation of protein quality are still appearing in the literature. Ad libitum or paired feeding, the level at which the test protein is to be fed, weight gain or protein gain, growth or composition of weight increment—these are a few of the questions which have been hotly debated.

F. S. Sherwood and V. Weldon (*J. Nutrition* **49,** *153 (1953)*) have compared four feeding methods useful in the evaluation of protein quality for the rat. With the ad libitum and the paired feeding methods, a 12 per cent protein diet was employed. A third feeding method, the constant protein procedure, involved feeding 4 g. of the protein test diet to each of the experimental animals along with as much of an energy supplement as would be eaten in twenty-four hours. The fourth method, called the adjusted protein method, was similar to the constant protein procedure, except that the amount of protein-containing diet was progressively increased as the animals gained weight. The weanling rats employed in the study were selected with extraordinary care.

The proteins compared in the study were (1) casein and (2) the proteins contained in cowpeas. Thus the casein-containing diet was essentially of the highly purified variety, while the other diet contained over 60 per cent cowpeas with corresponding adjustments in the other ingredients of the ration. It might be wished that the authors had chosen for comparison two purified proteins, so that the remainder of the diet might have been kept constant. As it is, the feeding of two such widely differing rations raises the question of palatability, an issue which could have been largely avoided. Furthermore, the diets used in the ad libitum and paired feeding experiments differed somewhat in composition from those used in the constant protein procedure and in the adjusted protein method. The differences are, however, small, and probably not significant. The energy supplement used in the constant protein procedure and in the adjusted protein method consisted of 10 per cent fat and 90 per cent carbohydrate.

The greatest difference in weight gain between groups receiving the two kinds of proteins occurred with the ad libitum feeding method, while the paired feeding method gave the least variability. The authors argue quite rightly that minimal variability is desirable, but that maximal differences between experimental groups are equally important. They selected as a criterion for the choice of feeding method the ratio of the difference in weight gain to the standard deviation of this difference. On this basis the ad libitum feeding method proved to be the most desirable, with the adjusted protein method ranking as second choice. The paired feeding technic was the least desirable. When the results of the experiments were calculated in terms of protein efficiency, the ratios of difference between experimental groups and standard deviation of that difference were so nearly alike for the four feeding methods that the authors concluded there was no clear-cut evidence for the superiority of any of the four methods.

In another paper, G. Litwack *et al.* (*J. Nutrition* **49,** *579 (1953)*) describe further work with a somewhat different approach to the testing of protein quality. In principle the method is based on changes in liver xanthine oxidase activity with quality of the protein fed. Diets containing casein, gliadin, or gliadin with amino acids were employed in this study. Xanthine oxidase was determined manometrically after the animals had been raised on the experimental ration. The liver xanthine oxidase level of casein-fed animals was higher than that of gliadin-fed rats. Supplementation of the ration with glycine gave the expected increase in biologic values. Rough

agreement exists between oxidase levels and growth response on the various diets studied. The method is reminiscent of an older one determining protein quality by measuring the rate of liver regeneration after partial hepatectomy.

C. M. Lyman, W. Y. Chang, and J. R. Couch (*J. Nutrition* **49,** *679 (1953)*) have devised a chemical method for the evaluation of the protein quality of cottonseed meal. Two properties of the cottonseed meal were found to be related to its protein value, (1) solubility in dilute sodium hydroxide, and (2) the concentration of gossypol. A chemical index whose numerator is per cent nitrogen solubility in dilute base and whose denominator is per cent total gossypol present was found to be in good agreement with the growth response of chicks. While such a method would be applicable only to specific problems, it could be extremely useful in limited cases.

Measurement of nitrogen balance has long been a useful device in the study of protein quality. However, with small animals such as the mouse food spillage has been a serious technical problem, since this renders difficult the precise measurement of nitrogen intake and in addition contaminates urine and feces with nitrogen-containing food. One solution to this problem has been to feed nitrogen-free rations, administering the protein or protein derivative by stomach tube. This procedure is not only tedious, but by its very nature limits the kind and the amount of protein which can be used. In this connection a paper by A. P. Martin, C. R. Shaw, and H. H. Williams (*J. Nutrition* **49,** *163 (1953)*) is especially noteworthy. These authors demonstrated that the rat was capable of utilizing intact heterologous proteins such as raw egg albumin, or bovine plasma when administered intraperitoneally. Young male rats past the period of most active growth were employed in these studies. One group received the protein-free diet with egg albumin or bovine plasma added to the ration. The other group received the protein-free diet with the protein administered intraperitoneally. When egg albumin was employed, the animals receiving this substance with the diet grew faster than did those which received it by injection. With the bovine plasma, however, no difference in weight gain for the two methods of administration could be detected. The animals were in positive nitrogen balance throughout the twelve weeks of the experiment. No signs of toxicity were detected in the injected animals.

Special problems in the determination of protein quality justified the use of special technics. In ordinary situations, however, there is still much to be said for the simple ad libitum technic using weight gain as the chief criterion. The simplicity of this method should not militate against its use.

REVERSAL OF AMINOPTERIN TOXICITY IN THE RAT

Aminopterin (4-aminopteroylglutamic acid) is the most potent of the folacin antagonists. The administration of relatively small amounts of this material to animals produces the symptoms of severe folacin deficiency. Many observations have shown that the toxic effects of aminopterin are not readily reversed by folacin. With small dosages of aminopterin relatively large amounts of folacin may prevent the toxic effects of the antagonist; also, treatment of animals with large doses of folacin prior to aminopterin administration may reduce the severity of the toxic manifestation.

The discovery of the *Leuconostoc citrovorum* factor by H. E. Sauberlich and C. A. Baumann (*J. Biol. Chem.* **176,** *165 (1948)*) helped clarify the aminopterin-folacin relationship. The chemical nature and some of the biologic effects of the citrovorum

factor have been reviewed (*Nutrition Reviews* **8,** *282 (1950)*). Aminopterin toxicity in microorganisms and animals is readily reversed by citrovorum factor. The observations of C. A. Nichol and A. D. Welch (*Proc. Soc. Exp. Biol. Med.* **74,** *52 (1950)*), which show that aminopterin inhibits the conversion of folacin to citrovorum factor, afford a plausible explanation for the noncompetitive nature of the aminopterin-folacin relationship and the competitive nature of the aminopterin-citrovorum factor relationship. There is a growing conviction among workers in the field that citrovorum factor represents the active metabolic product of folacin.

The recent report of H. E. Sauberlich (*J. Nutrition* **50,** *101 (1953)*) presents data concerning the relative effectiveness of citrovorum factor and folacin in reversing the toxic effects of aminopterin in rats. Sauberlich fed weanling rats a basal diet of casein, 20 g.; corn oil, 4 g.; cod liver oil, 1 g.; sucrose, 70.5 g.; salts, 4 g.; L-cystine, 0.3 g.; choline chloride, 0.2 g.; with the following vitamin supplements, alpha-tocopherol, 2.5 mg.; alpha-tocopherol acetate, 2.5 mg.; 2-methyl-1,4-naphthoquinone, 0.5 mg.; inositol, 100 mg.; niacin, 2 mg.; calcium pantothenate, 3 mg.; thiamine, 0.6 mg.; riboflavin, 0.6 mg.; pyridoxine, 0.6 mg.; folacin, 0.1 mg.; biotin, 0.05 mg.; and vitamin B_{12}, 0.003 mg. Animals were fed this basal diet with various combinations of aminopterin, folacin (pteroylglutamic acid), citrovorum factor (CF), and antibiotics. The citrovorum factor used was a synthetic product with the trade name leucovorin. In addition, some animals received intraperitoneal injections of folacin, CF, vitamin B_{12}, ascorbic acid, or cortisone. The animals were fed the diets for three to four weeks and observations were made on the survival, growth, hematologic findings, ascorbic acid concentrations in tissues, and the formation of methionine by liver tissue *in vitro*.

In the first experiments aminopterin was fed at levels of 0.75 and 1 mg. per kilogram of diet. The effects of daily intraperitoneal injections of folacin, CF, or vitamin B_{12} were studied. There were 6 to 25 animals per group except for the group receiving vitamin B_{12} which contained only 3 animals. When aminopterin was fed at a level of 0.75 mg. per kilogram of diet growth was severely depressed and only 40 per cent of the animals survived two weeks, none survived three weeks. The daily injection of 40 micrograms of folacin into these animals improved survival, with 5 of the 6 animals surviving the four-week experimental period. These injections of folacin did not restore growth to normal. In contrast, the daily injection of 40 micrograms of CF into animals fed 0.75 mg. of aminopterin per kilogram afforded complete protection as judged by growth and survival. When animals were fed 1 mg. of aminopterin per kilogram of diet only 5 of the 25 survived two weeks and none survived three weeks. Under these conditions of aminopterin feeding the daily injection of up to 250 micrograms of folacin (the highest level studied) resulted in no significant protection from the aminopterin toxicity. The daily injection of 25 or 50 micrograms of CF into animals receiving 1 mg. of aminopterin per kilogram of diet resulted in partial protection. These treatments permitted four-week survival rates of 4 out of 6 and 6 out of 8 animals respectively. Neither of these levels restored normal growth. The daily injection of 100 micrograms of CF afforded full protection for the animals receiving 1 mg. of aminopterin per kilogram of diet. Three of the animals receiving this level of aminopterin were injected with 10 micrograms of vitamin B_{12} daily. Under these conditions vitamin B_{12} had no effect on the aminopterin toxicity.

In the second series of experiments aminopterin was fed at a level of 1 mg. per kilogram of diet and the supplements of CF and folacin were added to the diet. The same toxicity of aminopterin was noted with only 3 of the 24 animals surviving two weeks.

As little as 1 mg. of CF per kilogram of diet afforded partial protection, with 5 of the 10 animals surviving the three-week experimental period. The feeding of 5 mg. of CF per kilogram of diet permitted survival of all the animals; however, growth was not completely restored. Ten mg. of CF per kilogram of diet afforded complete protection. Under the same conditions of aminopterin feeding 10, 25, or 50 mg. of folacin per kilogram of diet afforded only partial protection. The feeding of 100 mg. of folacin per kilogram of diet completely protected the animals from the aminopterin toxicity.

In other experiments groups of rats receiving 1 mg. of aminopterin per kilogram of diet were injected with 200 micrograms daily of cortisone acetate or 10 mg. daily of ascorbic acid, or were fed 100 mg. per kilogram of diet of penicillin or aureomycin. None of these supplements afforded any protection from the aminopterin toxicity.

Data are given for peripheral leukocytes, erythrocytes and hemoglobin concentration for animals fed 1 mg. of aminopterin per kilogram of diet and the diet with various supplements of CF and folacin. Aminopterin feeding resulted in a severe leukopenia and anemia. Of particular interest is the severity of the anemia observed after only eight days of aminopterin feeding. The peripheral erythrocyte count was less than 2 million cells per microliter of blood. When one considers that the average life span of the red blood cell in the rat is approximately one hundred days (N. I. Berlin, R. L. Huff, and T. G. Hennessy, *J. Biol. Chem.* **188,** *445 (1951)*) development of this degree of anemia in eight days must indicate that the aminopterin feeding resulted in the destruction of existing red blood cells. The author did not comment on this point, but it seems worthy of further study.

For the blood studies CF was injected intraperitoneally at levels of 25, 50 and 100 micrograms daily into the aminopterin-fed rats, and folacin was injected at a level of 250 micrograms daily. Statistical analysis of the results of these experiments would have been of great value. However, from the data given it is doubtful if any of these injections completely corrected the anemia and leukopenia of the aminopterin-fed rats. When the supplements were included in the diet 10 mg. of CF per kilogram of diet were required to prevent the anemia and leukopenia. The feeding of 20 mg. of folacin per kilogram of diet was ineffective, but 100 mg. per kilogram of diet afforded protection.

Studies were also made on the effect of aminopterin feeding and supplementation with CF and folacin on the formation of methionine by liver homogenates *in vitro*. The number of animals per group was quite small and no statistical evaluation of the data was made. The data presented indicate that aminopterin feeding leads to a reduction in the ability of liver tissue to methylate homocysteine. This aminopterin effect appeared to be prevented by the inclusion in the diet of 10 mg. of CF, or by 100 mg. of folacin per kilogram of diet.

It was also reported that aminopterin feeding led to a lowered concentration of ascorbic acid in the liver and that this reduction was prevented by the addition of 10 mg. of CF or 100 mg. of folacin per kilogram of diet. The number of animals used for these determinations was not stated.

From a consideration of all the data given it appears that when a rat is fed 1 mg. of aminopterin per kilogram of diet, between 5 and 10 mg. of citrovorum factor per kilogram of diet, or approximately 100 mg. of folacin per kilogram of diet are required to protect the animal. As pointed out by the author, the synthetic citrovorum factor (leucovorin) used in these experiments is probably only half as active as the naturally occurring products. Thus the naturally occurring citrovorum factor is probably twenty to forty times as effective as folacin in preventing aminopterin toxicity in the rat.

NOTES

Exchangeable Potassium in Diabetes

The metabolism of potassium in diabetes has received a great deal of attention since the classic work of D. W. Atchley *et al.* (*J. Clin. Invest.* **12,** *297* (*1933*)) who found that following the withdrawal of insulin there was a negative potassium balance as well as a negative phosphorus and nitrogen balance. The balances were restored by the readministration of insulin. Although the serum potassium concentration in diabetic acidosis may be high, normal, or even low under certain circumstances, it appears evident that in this condition there is a deficit of body potassium, presumably intracellular, because most of the potassium in the body is in this compartment (*Nutrition Reviews* **5,** *315* (*1947*); **7,** *296* (*1949*)).

J. K. Aikawa, J. H. Felts, and G. T. Harrell (*J. Clin. Invest.* **32,** *15* (*1953*)), using the radioactive isotope of potassium (K^{42}), have measured the exchangeable body content of potassium in a group of 42 diabetic subjects in various stages of control and with the common complications of diabetes, but, with one exception, not in acidosis or coma.

An initial measurement of exchangeable body potassium was made for each of the patients. In addition, some of the patients were given potassium by mouth (1 g. three times daily) for a week and a second determination was made. In many patients it was possible to make a third measurement a week after the second. The results were compared according to sex to values found previously by the authors in normal subjects, presumably by the same methods.

The results indicated a significant reduction in exchangeable potassium, in terms of milliequivalents per kilogram body weight, in the diabetic males as compared to normal males. However, in the females there was not a significant reduction in exchangeable potassium in the diabetic subjects. Inspection of the data reveals a very much wider range of exchangeable potassium in the female diabetic subjects than in the males. Moreover, in the female diabetic patients the range is so great compared to normal female subjects as to suggest at least a marked lability in body potassium content in the former. The reason for this sex difference in exchangeable potassium is not clear. Of the subjects in whom serial determinations of exchangeable potassium were made after potassium administration for a week, 7 increased their exchangeable potassium. This was considered to substantiate the existence of a deficit and occurred particularly in those in whom the control of diabetes was the poorest.

The authors concluded that adequacy of control of diabetes in their patients correlated, at least roughly, with the presence or absence of a body potassium deficit.

England's National Food Survey—1950

The *Bulletin of Hygiene* (**28,** *448* (*1953*)) has reviewed a report by the Ministry of Food entitled "Domestic Food Consumption and Expenditure, 1950, with a Supplement on Food Expenditure by Urban Working-Class Households, 1940–1949. Annual Report of the National Food Survey Committee." Data were collected from 4723 households representing rural homes, urban working-class homes and other urban households. In each the food consumed by every member of the household was weighed for a period of one week.

The *Bulletin of Hygiene* reviewer raises the question whether a survey of nearly 5000 homes was actually required or whether it represents in part a waste of the taxpayer's money. He calls attention to the fact that the picture presented in "Food, Health and Income" (*2d edition, Macmillan, London* (*1937*)) by Lord John Boyd Orr was derived from 1152 families and has never been seriously questioned.

Some of the conclusions reached in the 1950 report are by no means surprising, *i.e.*, that the amount of money spent per

capita on food increases with family income but decreases with the number of children in the family. However, the report indicates that there is no longer the close association between family income and the quality of food consumed that existed in prewar days. In fact, the diets reported are considered to be generally satisfactory. Whether the diets of the survey week are actually representative of the general picture or whether they reflect to some extent a desire to impress the interviewer is difficult to decide. According to the report, the narrowing of the gap between low and high income groups has been accomplished largely by a rise in the quantity and quality of food consumed by the poor and only to a small extent by the lowering of the standard of the high income groups. The report in general indicates that, from the nutritionist's point of view, England's limited food supply has been put to commendably good use.

Aging, Vascular Disease and Cerebral Circulation in Man

The current interest in the aging process has brought into clear focus the necessity for differentiating the process of natural senescence from that of the chronic diseases so often seen in aging individuals such as hypertension, diabetes, arteriosclerosis, and others. Whether the process of senescence itself can be slowed or otherwise altered is one matter, but the prevention, pathogenesis and treatment of vascular disease, for example, is quite another and perhaps, in the present state of our knowledge, more amenable to preventive and therapeutic measures.

H. A. Shenkin *et al.* (*J. Clin. Invest.* **32,** *459 (1953)*) have used methods developed in their laboratory for measuring total cerebral blood flow and cerebral oxygen consumption. These observations were made on individuals showing aging, arteriosclerosis, or hypertension alone, or combinations of these. Their subjects were divided into five groups: (1) individuals without arteriosclerosis or hypertension and under 40 years of age; (2) individuals above 50 years of age with evidence of arteriosclerosis but without hypertension; (3) individuals with hypertension and arteriosclerosis above 40 years of age, but without evidence of cerebral vascular accident or mental deterioration as complications of hypertension or arteriosclerosis; (4) the same as group (3), but with evidence of cerebral vascular accidents or mental deterioration; (5) individuals with hypertension alone without reference to age and without arteriosclerosis. The results of these studies need not be discussed in detail. There seemed to be evidence, however, that aging per se (mean age 68) did not alter the cerebral blood flow or oxygen consumption from the normal. In contrast, hypertension seemed to be the most important factor in reducing these two measurements, although as might be expected it was not always easy to separate arteriosclerosis from hypertension since the former often accompanies the latter. Of further interest was the authors' failure to find that cerebral vascular accidents or mental deterioration altered the cerebral blood flow from that of the group without cerebral vascular accidents.

The authors note that cerebral oxygen consumption was found in conscious, alert individuals to be even below that usually found in diabetic coma or pentothal narcosis, pointing up the difficulty of correlating cerebral blood flow and states of consciousness.

The evidence presented that aging per se does not necessarily reduce the cerebral blood flow or oxygen consumption points again to the need for intensive study of arteriosclerosis and hypertension, and their prevention. It is hardly necessary to state that these two diseases are intimately related to nutrition with respect to their etiology and treatment.